Art. XVIII.—*Some Anatomical and Cytological Studies on Fiji Disease of Sugar Cane.*

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Fiji disease of sugar cane is considered on the evidence available to be one of the virus group of plant diseases, but differs from all other plant viruses in that the stunting of the plant is accompanied by the occurrence of galls in the stem and leaf, but not, as far as the writer has observed, in the root. McWhorter(.5) reports root galls, but in a large number of roots of Fiji diseased cane examined by the present writer no galls were found.

I. Appearance of the Gall.

Stem galls cause no abnormality in the external appearance of the plant, but, on sectioning, it can be seen by the naked eye that many of the vascular bundles are larger than normal.

Leaf galls, however, protrude on the abaxial surface of the leaf, run parallel with the midrib, and are obviously continuations of individual vascular bundles. Occasionally, two adjacent bundles will form parallel galls, but there is always a line of demarcation showing from which bundle the gall is derived.

The galls therefore are purely vascular, and the course of the vascular bundle is not altered by gall formation. The non-vascular parenchyma is not influenced by gall formation, except that the cells are rather smaller than normal. The number of chloroplasts does not appear to be reduced, so that the darker green colour that is characteristic of the diseased leaves is apparently due to the crowding of the chloroplasts within the cells. The deformation of the leaves is due to the proliferation of cells in the vascular bundles causing a twisting of the parts of the leaf which are not hypertrophied. The dwarfing is no doubt due to the derangement of the metabolic functions consequent on gall formation where the vascular tissues are involved.

II. Anatomical Structure of the Gall.

A description of the anatomy of the healthy sugar cane leaf is omitted in this paper as it is given fully in a paper by Artschwager (2) and need not be repeated here.

There are five regions in the gall (Fig. 1A) :—

    1. Protoxylem vessels and lacuna as in healthy tissues.

2. Primary metaxylem vessels, as in healthy tissues, though these may be more or less hypertrophied. In some cases, projections of the lignified cell walls have been observed.
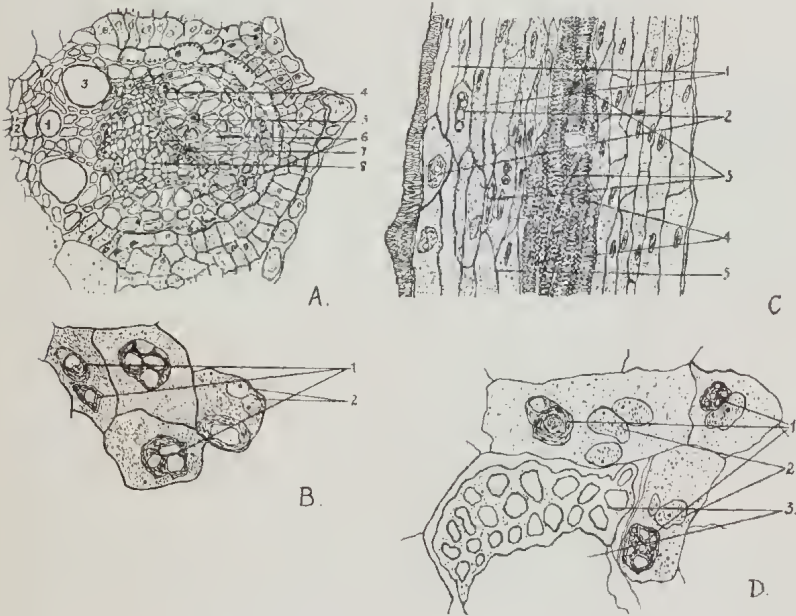

Fig. 1.—A. Transverse section of a gall, showing hypertrophied phloem and xylem, and the position of the pseudoparenchyma adjacent to the pseudotracheids. 1. Protoxylem vessels; 2. Protoxylem lacuna; 3. Primary metaxylem; 4. Partially lignified cell still containing nucleus; 5. Isolated thin walled pseudoparenchyma cell; 6. Pseudotracheidal tissue; 7. Pseudoparenchyma tissue; 8. Phloem. B and D. Pseudoparenchyma cells containing " X " bodies. 1. " X " bodies; 2. Multinucleate tissue; 3. Pseudotracheid. Note that three cells in B have " X " bodies and no nucleus, and all the cells in D contain both bodies and nuclei. C. Longitudinal section through portion of a gall showing stages in the formation of pseudotracheidal tissue. 1. Sieve tubes; 2. Enucleate cells containing " X " bodies, one showing partial thickening of the cell wall; 3. Pseudotracheids; 4. Pseudotracheids containing " X " bodies; 5. Pseudoparenchyma.

3. The " sclerotic cells " of Kunkel (3) and Lyon (4), for which the writer suggests the more definite name of " pseudotracheid." These are lignified and pitted cells forming a tracheidal tissue which more or less surrounds the bundle, and groups of these cells may be interspersed among the phloem and parenchyma cells. They have no definite orientation, and are often branched. Their reticulately pitted or scalariform walls can be seen in transverse as well as in longitudinal section. They differ from the sclereides

of the polar caps of normal bundles in size, shape and pitting, and such sclereides are absent in gall tissue. This difference is especially noticeable in the leaf, where the sheath is normally composed of collenchyma with stratified and pitted walls. These pseudo-tracheids are arranged roughly parallel with the axis of the leaf, and true vessels are sometimes scattered through them.

4. A hypertrophied phloem, which, in leaf galls, may consist of over 100 sieve tubes and companion cells, and may form rays which project into the pseudotracheidal tissue and beyond it. An extreme case showed the phloem lying external to the sclerotic tissue in a stem bundle. The phloem of a normal macro-bundle in the leaf has from 16 to 32 sieve tubes, as determined from a number of counts.

5. The pseudoparenchymatous tissue, which is irregularly placed, but which lies in the main between the phloem proper and the sclerotic tissue, consists of equi-diametrical cells with large and rapidly dividing nuclei. These are the cells which contain the Fiji or "X" bodies, known hitherto as *Northiella sacchari*, Lyon. These cells can be traced to give rise to the pseudotracheids and to sieve tubes, and radiate through these tissues more or less, without definite orientation. At times, a lone pseudoparenchyma cell will be observed in the pseudotracheidal tissue, with its large nucleus and unthickened cell wall (Fig. 1A, 5). These cells appear to form pseudotracheids by secreting a lignified cell wall, later lose their nuclei often show "X" bodies, and lastly lose their cell contents. Stages in this transformation are shown in Fig. 1, c and D. We thus find masses of phloem surrounded more or less completely by sclerotic tissue, and, at times, pseudotracheids surrounded by phloem.

In the stem, these gall bundles displace, and, at times disrupt the parenchyma of the stem, and their orientation, especially in the peripheral region, is very varied. They are characteristically asymmetrical, there being much more development of certain tissues on one side of the bundle than on the other.

The writer has not found, in any of the literature on this disease any mention of galls in the micro-bundles of the leaves, and they are likely to be overlooked. They are, however, characteristically present, and result in a hypertrophy of the phloem, the presence of a pseudoparenchyma consisting of a few cells, and the deformation and asymmetry of the xylem. Thus, the development of galls is not so localized as might be

imagined from a cursory examination, and hypertrophy occurs throughout the vascular tissue of the stem and leaf, which accounts for the very great deformation thereof . in the later stages of the disease.

The chlorenchyma and epidermis on the abaxial side of the bundles may be disrupted in old galls.

Kunkel (3) gives the following description of the formation of the pseudotracheids :—" After a gall reaches a certain stage of maturity, a curious change takes place in the surrounding tissues. These tissues are derived from the cells that would normally produce the sclerenchymatous sheath, and, in some instances from portions of the phloem. Many of the cells of this tissue enlarge, and take on the staining reactions and appearance of tracheids. Their walls become thickened and lignified. Most of the thickening occurs as reticulate fibrous bands. The cells retain their shape, but become hard and woody."

In discussing the seat of the disease in the tissues, Kunkel states :—The galls of Fiji disease always originate in the phloem and, although the disease affects other tissues to a certain extent it must be regarded as a phloem disease."

This is not in accordance with the present findings, for the pseudotracheidal tissues are as characteristic of the disease as the hypertrophied phloem, and so also are the undifferentiated cells. The writer considers that the cells containing the bodies, and the nucleate pseudoparenchyma are a persistent cambial meristem, which differentiates into a phloem and a false xylem, and that the seat of the disease is in this tissue, i.e., the disease is essentially meristematic, and causes a new growth comparable with that seen in carcinomatous sections of animals. The distribution of these cells with their large nuclei resembles that in carcinoma, as does the rapidity of their division. The occurrence of inclusion bodies cannot be paralleled in animal cancer, but is common in plant virus diseases. We cannot therefore push the analogy too far, but it is certainly worth noting.

In maintaining the theory of meristematic infection for Fiji disease, the findings of Arber (1) are of interest. She gives cases of the formation of a secondary metaxylem in mono-cotyledonous bundles in the Araceae. This takes place by means of a persistent cambial tissue, which is not characteristic of the monocotyledons as a group. The result is the formation of an amphivasal bundle as she figures. The general appearance of the bundle is similar to that of the Fiji gall, except that the distribution of the tissue is more uniform in the former. Phloem parenchyma is not normal in sugar cane, except in the growth ring region. The infective agent of Fiji disease appears to cause the persistence and proliferation of the cambium tissue,

or pseudocambium, for the cells are not quite typically cambi-
form. Their position is usually between the primary metaxylem
and phloem in young galls, but later between the secondary
metaxylem, and the phloem. They appear to give rise to phloem
and xylem elements abnormally. This theory, derived from the
anatomical evidence fits in well with the physiological evidence.

III. Facts in Favour of Meristematic Infection.

(*a*) In Fiji diseased tissue the sieve tubes and companion
cells are more numerous than in normal tissue, which suggests
cambial activity.

(*b*) The pseudotracheids are, as serial sections of the galls
show, produced by a metamorphosis of the pseudoparenchyma,
which is certainly meristematic, as is observed by Kunkel.

(*c*) The sclerotic tissue is homologous with a secondary
metaxylem. That this is so is confirmed by the study of the
galls in the peripheral bundles of the stem, and the micro-bundles
of the leaf. In the case of the former, the sclerenchyma sheath
still exists in part, though it is less regular, and less developed
than in normal tissue. The wood, instead of consisting of
one to three definitely and symmetrically arranged vessels, is
formed of a number of lignified elements which may divide the
phloem into two parts. In this case, the pseudotracheids are
continuous with the metaxylem. The pseudoparenchyma is asso-
ciated with the phloem and pseudotracheidal tissue.

(*d*) In the earlier stages of the formation of the pseudo-
tracheids, Northiella bodies are present in the partly thickened
cells, but, later, the nucleus, cell contents and bodies disappear.

(*e*) Rays of sieve tubes frequently cut through the sclerotic
sheath, and the phloem may lie outside this in the stem galls.
There is no constant arrangement of the various tissues in a gall.
The frequent occurrence of true vessels in the pseudotracheidal
sheath strongly supports the assumption that this is a secondary
xylem. In the peripheral stem bundles, tracheids may and do,
at times, replace the vessels in healthy cane (Artschwager 2),
and in Fiji diseased tissue, the peripheral bundles have, instead
of one or two vessels or tracheids, a series of pseudotracheids
as described above, arranged irregularly to the protoxylem, if
this is present. Frequently, it is absent.

(*f*) The fact that this xylem is continuous with the pseudo-
tracheids, and that pits occur between the two, strongly supports
the theory, since pits are absent in the cells adjacent to normal
vessels. In the very young galls, hypertrophy of the metaxylem
is the first symptom, and later sclerotic patches are formed.
The sclerotic cells, in leaf, and stem galls of the central type
usually form a tissue surrounding the phloem more or less com-
pletely, but patches of this tissue may be isolated from the rest.
and while the amphitracheidal type is commonest, there is con-
siderable variation, as is the case with the majority of galls.

IV. Cytology of the Pseudoparenchyma.

In 1910, Lyon (4), published the first record of Fiji disease, in which he noted the presence of bodies in the gall parenchyma, and thought they were of a plasmodial nature. Subsequently Lyon (4), in collaboration with North, carried out some cytological studies on the disease, and later named the supposed parasite *Northiella sacchari*. He describes the bodies as being " usually vacuolate, and appearing to have the structure of dense protoplasm." He failed to find a definite nucleus, though, he asserts "a body resembling this was sometimes present." McWhorter (5) claimed some success in the cultivation *in vitro* of these bodies, but the writer was unable to repeat his results. It appears more probable that these inclusion bodies are homologous with those occurring in other plant virus diseases, and a study of the gall tissues strongly suggests that they are formed during the degeneration of the pseudoparenchyma cells into pseudotracheids.

From a study of a number of living and stained sections, the former mounted in isotonic sugar solution, it appears that the mononucleate pseudoparenchyma cell frequently becomes multinucleate, inclusion bodies appear, the nucleus disappears, as thickening of the cell wall occurs, and finally the bodies and cell contents disappear, and a pseudotracheid is formed (Fig. 1, B, C, and D). Further, from the fact that the bodies contain strongly chromatic material, and are much more strongly stained by nuclear stains than the nuclei themselves, the theory that they are composed of degenerating nuclear material becomes attractive.

V. Summary.

1. The vascular nature of the infection in Fiji disease is shown.

2. It is suggested that the seat of the disease is in a persistent cambium, and not in the phloem as previous authors have asserted.

3. The nature of the inclusion bodies is discussed.

VI. References.

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